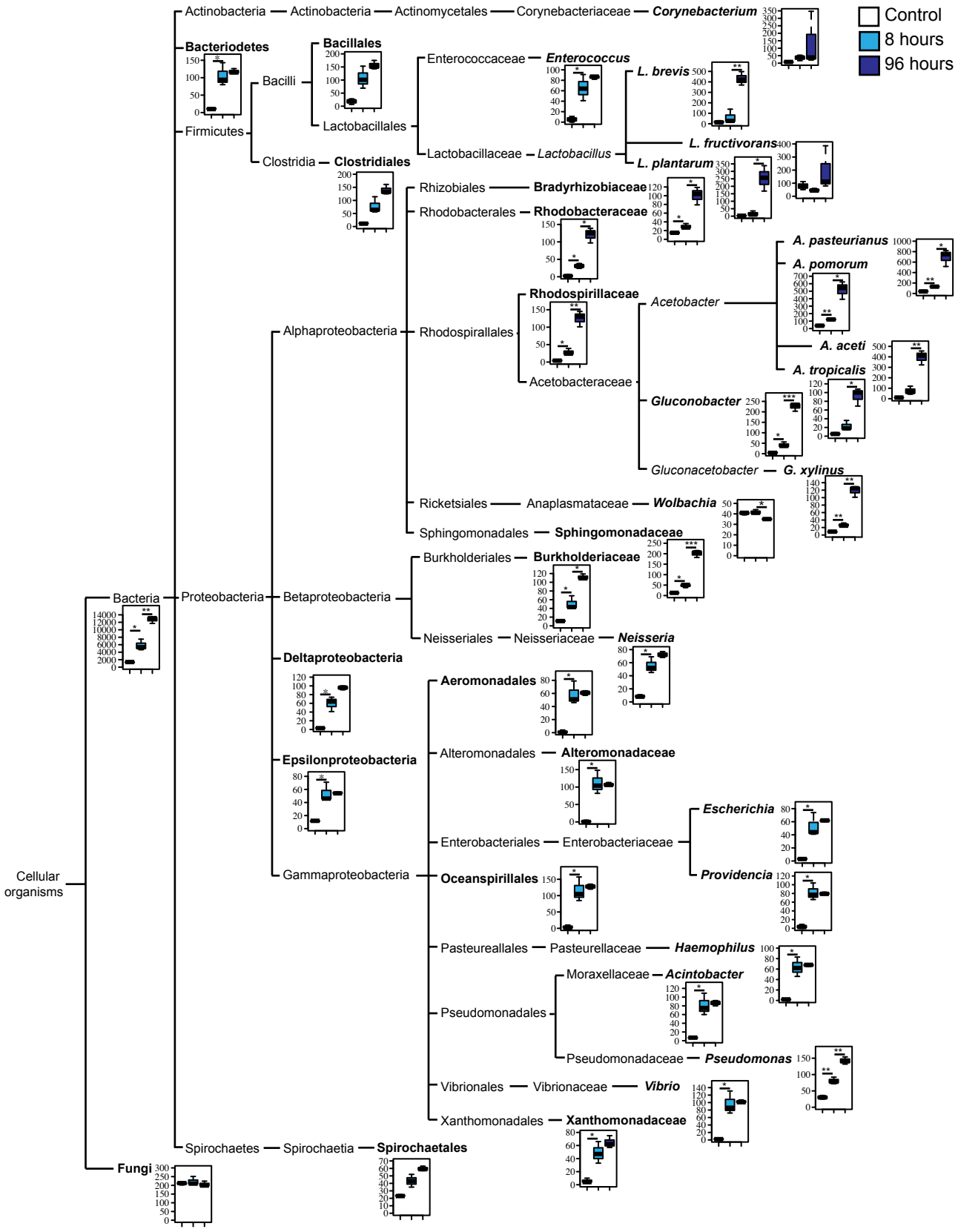
Control
8 hours
96 hours
Cellular organisms
Bacteria
Fungi
Proteobacteria
Actinobacteria
Actinobacteria
Actinomycetales
Corynebacteriaceae
Corynebacterium
Firmicutes
Bacilli
Bacillales
Lactobacillales
Enterococcaceae
Enterococcus
L. brevis
L. fructivorans
Lactobacillaceae
Lactobacillus
L. plantarum
Clostridia
Clostridiales
Bacteroidetes
Alphaproteobacteria
Rhizobiales
Bradyrhizobiaceae
Rhodobacterales
Rhodobacteraceae
Rhodospirillales
Rhodospirillaceae
Acetobacteraceae
Acetobacter
A. pasteurianus
A. pomorum
A. aceti
A. tropicalis
Gluconobacter
Gluconacetobacter
G. xylinus
Ricketsiales
Anaplasmataceae
Wolbachia
Sphingomonadales
Sphingomonadaceae
Betaproteobacteria
Burkholderiales
Burkholderiaceae
Neisseriales
Neisseriaceae
Neisseria
Deltaproteobacteria
Epsilonproteobacteria
Gammaproteobacteria
Aeromonadales
Alteromonadales
Alteromonadaceae
Enterobacteriales
Enterobacteriaceae
Escherichia
Providencia
Oceanspirillales
Pasteureallales
Pasteurellaceae
Haemophilus
Pseudomonadales
Moraxellaceae
Acintobacter
Pseudomonadaceae
Pseudomonas
Vibrionales
Vibrionaceae
Vibrio
Xanthomonadales
Xanthomonadaceae
Spirochaetes
Spirochaetia
Spirochaetales

Figure S1, related to Figure 1. Bacterial population composition following intestinal barrier dysfunction. Tree representing the taxonomic positions of the bacterial taxa identified in our metagenomic sequencing analysis. Only taxa with abundance similar to, or greater than, the *Drosophila* endosymbiont *Wolbachia* are shown. Taxa at terminal branches are highlighted in bold. Normalized numbers of sequence assignments at 8 and 96 hours post-barrier dysfunction, and in controls, are shown only for the terminal branches, and represent a readout of taxon abundance. n = 3 replicates of 5 dissected intestines from 30-35 day old w^{1118} female flies. Boxplots display the first and third quartile, with the horizontal bar at the median. * p<0.05, ** p<0.01, *** p<0.001, t-test.

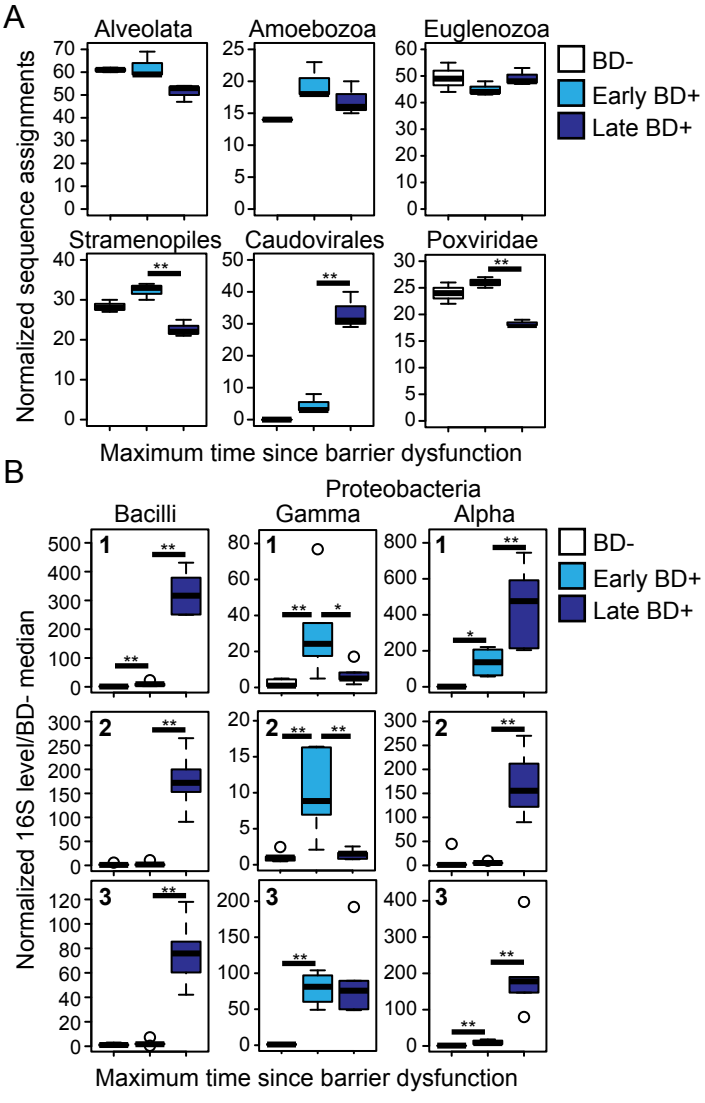

Figure S2, related to Figure 1. Commensal population composition following intestinal barrier dysfunction. (A) Normalized number of sequence assignments as a readout of taxon abundance at 8 and 96 hours post-barrier dysfunction, and in controls (BD-). Showing non-fungal eukaryote, and viral taxa. n = 3 replicates of 5 dissected intestines from 30-35 day old w^{1118} female flies. (B) Bacterial levels assayed by taxon specific qPCR of the 16S ribosomal RNA gene within 8 and 96 hours following barrier dysfunction (BD+), and in non-Smurf controls (BD-). Data from three independent experiments are shown (labeled 1-3). n = 6 replicates of 5 30-35 day old w^{1118} female flies. Boxplots display the first and third quartile, with the horizontal bar at the median. * p<0.05, ** p<0.01, *** p<0.001, t-test.

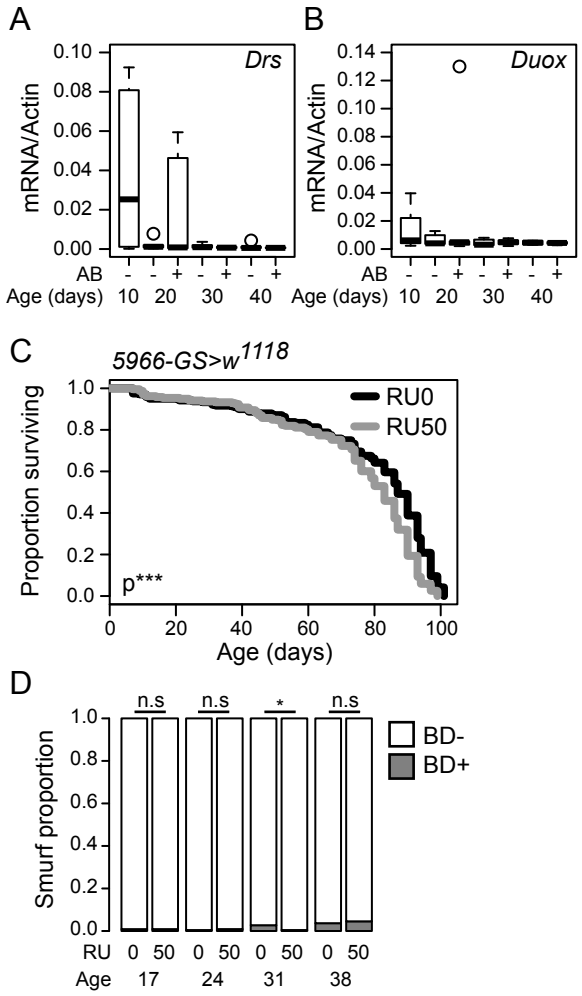

Figure S3, related to Figure 3. Intestinal immune activation shortens life and induces intestinal barrier dysfunction, but may not promote age-onset barrier failure. (A-B) Gene expression assayed by qPCR from dissected intestines of untreated (AB-) or antibiotic treated (AB+) w^{1118} female non-Smurfs at 10 day intervals. Antibiotic treatment was from 10 days of age. n = 6 replicates of 5 intestines. *Drosomycin (Drs)* (A), *Dual Oxidase (Duox)* (B). (C-D) Lifespan curves (C) and Smurf proportions (D) of w^{1118}/5966-Geneswitch female flies drug fed from day 10 of adulthood (RU50), and carrier fed controls (RU0), Smurfs were counted weekly from 7 days following induction of overexpression. n = >200 flies. BD- = non-Smurf, BD+ = Smurf. Boxplots display the first and third quartile, with the horizontal bar at the median. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Log Rank test for survival data, Binomial test for Smurf proportions, Wilcoxon test for other data.

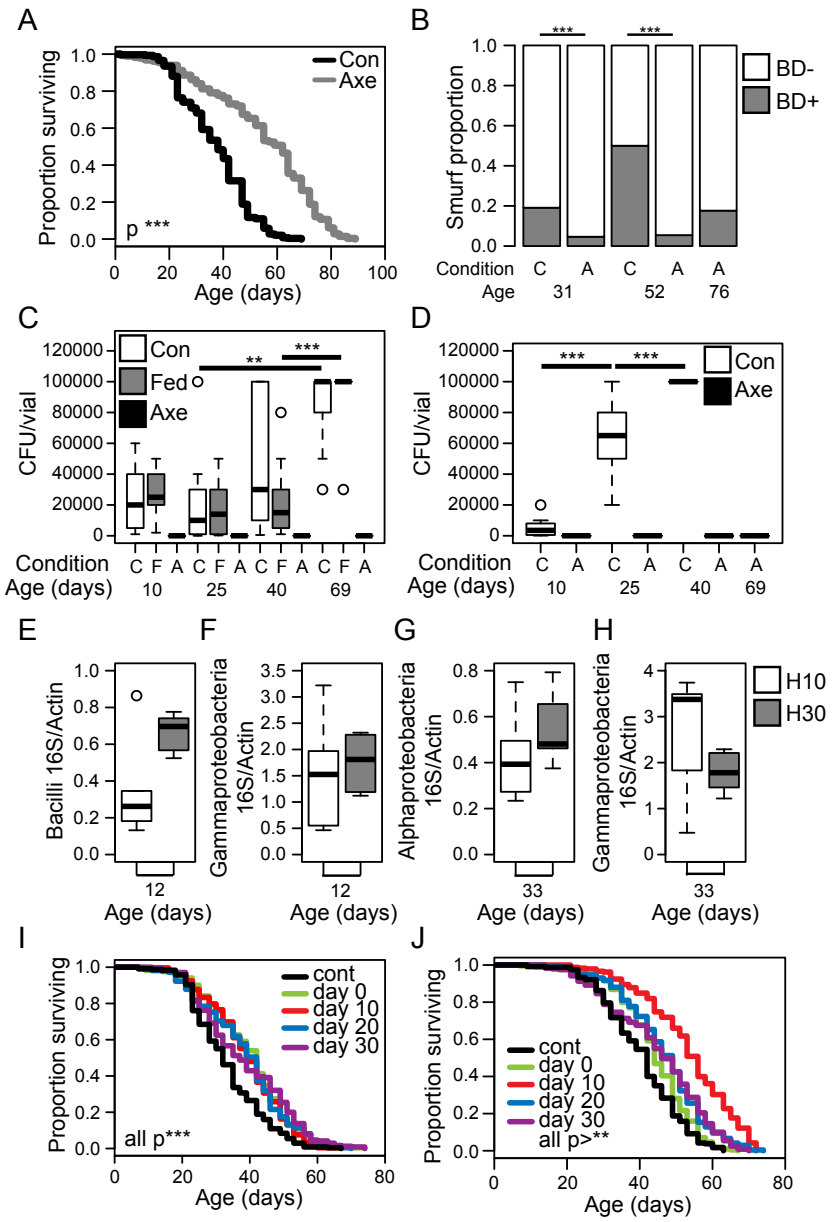

Figure S4, related to Figure 4. The early life microbiota regulates lifespan and intestinal barrier failure. (A and B) Lifespan curve (A) and Smurf proportions (B) of Canton S female flies conventionally and axenically reared. n = >250 flies/condition. C – conventional, A – axenic. (C) Colony forming units from vials supporting w^{1118} female flies conventionally reared, axenically reared, and axenically treated and exposed to fly homogenate as embryos. n = 10 vials/condition. (D) Colony forming units from vials supporting Canton S female flies conventionally and axenically reared. n = 10 vials/condition. (E-H) Bacterial levels assayed by taxa specific qPCR of the bacterial 16S ribosomal RNA gene in w^{1118} females fed homogenate from 10 day old or 30 day old flies at the 10 day timepoint. (E and F) Bacilli (E) and Gammaproteobacteria (F) levels at 12 days of age, (G and H) Alphaproteobacteria (G) and Gammaproteobacteria (H) levels at 33 days of age. n = 6 replicates of 5 surface sterilized whole flies. (I) Lifespan curve of antibiotic treated w^{1118} female flies, and untreated controls. Antibiotic treatment was started at the indicated age. n = >200 flies/condition. P-values are compared to untreated controls. (J) Lifespan curve of antibiotic treated Canton S female flies, and untreated controls. Antibiotic treatment was started at the indicated age. n = >200 flies/condition. P-values are compared to untreated controls. Boxplots display the first and third quartile, with the horizontal bar at the median. * p<0.05, ** p<0.01, *** p<0.001, Log Rank test for survival data, Binomial test for Smurf proportions, Wilcoxon test for other data.

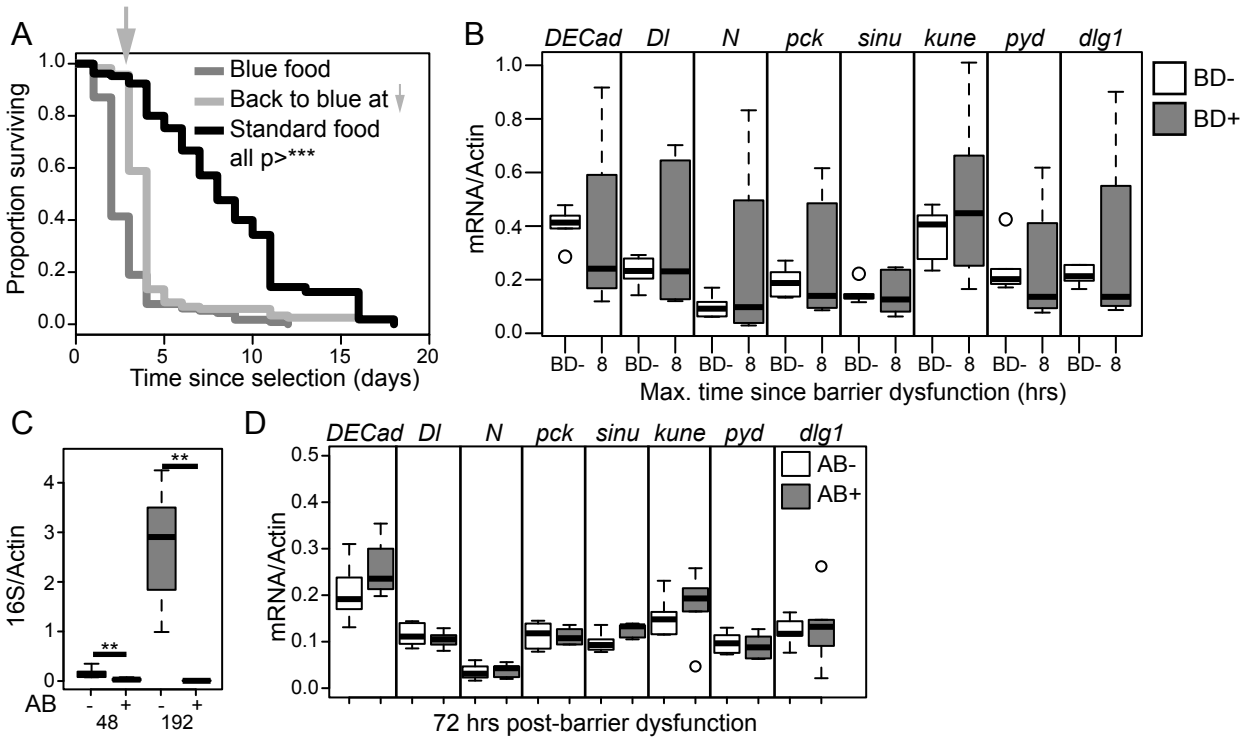

Figure S5, related to Figure 5. Loss of barrier function is progressive and accompanied by dysbiosis-dependent changes in intestinal and excretory function. (A) Survival curves of w^{1118} female 24 hour post-Smurf flies, 30 days old at selection. Flies were either removed from the blue dye to standard food, retained on the blue food, or initially removed and allowed to clear the blue dye and then returned to the blue food at 72 hours post-Smurf. n = >150 flies/condition. P-values are compared to the standard food condition. (B) Junction protein gene expression assayed by qPCR from dissected intestines of 30 day old w^{1118} female non-Smurfs, and 8 hours post-Smurf. n = 6 replicates of 5 intestines. (C) Bacterial levels assayed by qPCR of the bacterial 16S ribosomal RNA gene in 30-35 day old w^{1118} female Smurfs fed antibiotics from 24 hours post-Smurf, and untreated controls. Data is shown from 48 hours and 192 hours post-Smurf. n = 6 replicates of 5 intestines. (D) Junction protein gene expression assayed by qPCR from dissected intestines of 30-35 day old w^{1118} female 72 hour post-Smurf flies fed antibiotics from 24 hours post-Smurf and untreated controls. n = 6 replicates of 5 intestines. *DECad – Drosophila E-Cadherin, Dl – Delta, N – Notch, pck – pickle, sinu – sinuous, kune – kune-kune, pyd – polychaetoid, dlg1 – discs large 1*. Boxplots display the first and third quartile, with the horizontal bar at the median. * p<0.05, ** p<0.01, *** p<0.001, Log Rank test for survival data, Wilcoxon test for other data.

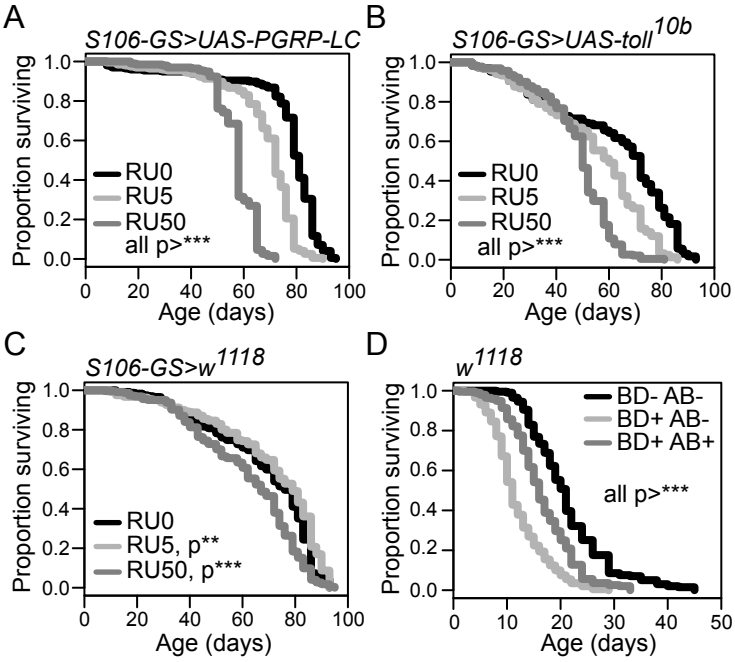

Figure S6, related to Figure 6. Preventing dysbiosis post-barrier dysfunction restores lifespan.

(A-C) Lifespan curves of female flies drug induced from day 10 of adulthood with a low (RU5) or high (RU50) dose, and uninduced controls (RU0), UAS-PGRP-LC/S106-Geneswitch (A), UAS-Toll^{10b}/S106-Geneswitch (B), w^{1118}/S106-Geneswitch (C). n = >200 flies/condition. (D) Survival curves of w^{1118} female 24 hour post-Smurf flies, 30 days old at selection, treated with antibiotics between 24 and 72 hours post-Smurf, untreated controls, and age-matched untreated non-Smurf controls. n = >200 flies/condition. P values are compared to non-Smurf controls. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, Log Rank test.

Table S1, related to Figure 1 and Figure S2. Summary of top 100 BLAST hits[a] for 16S rRNA gene clones generated from taxon specific and universal primer sets.

Primer	Seq. ID	# clones	# genera hit	Top hit genus (% hits)	% ID range	% query cover	E-value range	Score range	Megan assigned LCA[b]
Alpha	Con3	23	6	*Acetobacter (47)*	97-100	98-100	2e-59-3e-67	226-252	Acetobacteraceae
Gamma	Con4	23	38	*Orbus (1)*	83-96	59-100	7e-55-4e-32	135-211	Gammaproteobacteria
Bacilli	A6	1	4	*Lactobacillus (78)*	92-99	98-100	3e-90-1e-113	329-407	*Lactobacillus*
	B6	1	3	*Lactobacillus (92)*	93-99	98-100	3e-90-3e-110	329-396	*Lactobacillus*
	D6	1	3	*Lactobacillus (87)*	93-99	98-100	1e-88-9e-110	324-294	*Lactobacillus*
	F5	1	3	*Lactobacillus (87)*	93-99	98-100	1e-88-9e-110	324-294	*Lactobacillus*
	F6	1	3	*Lactobacillus (91)*	93-99	98-100	4e-88-4e-113	322-405	*Lactobacillus*
	G5	1	4	*Lactobacillus (80)*	92-99	98-99	3e-89-2e-112	326-403	*Lactobacillus*
	aA3	1	4	*Lactobacillus (92)*	93-99	97-99	6e-87-3e-110	318-396	*Lactobacillus*
	aF3	1	4	*Lactobacillus (92)*	94-97	100	2e-92-7e-106	337-381	Lactobacillaceae
	aG3	1	3	*Lactobacillus (91)*	94-100	98-99	6e-92-3e-114	335-409	*Lactobacillus*
	Con6	6	4	*Lactobacillus (80)*	92-99	99-100	4e-88-2e-111	322-399	*Lactobacillus*
	Con7	4	3	*Lactobacillus (91)*	93-100	99-100	2e-86-3e-109	316-392	*Lactobacillus*
	Con8	4	4	*Lactobacillus (91)*	92-100	98-100	5e-82-1e-108	302-390	*Lactobacillus*
Universal	G7	1	10	*Acetobacter (50)*	93-99	86-100	6e-113-1e-165	472-580	*Acetobacter*
	Con1	13	10	*Acetobacter (50)*	94-99	86-100	2e-132-3e-165	470-579	*Acetobacter*
	Con2	3	2	*Lactobacillus (86)*	84-99	70-100	5e-100-0	363-686	*Lactobacillus*
	Con5	6	3	*Lactobacillus (87)*	84-100	75-100	3e-96-0	350-658	*Lactobacillus brevis*

[a]Short amplicon lengths from these primer sets make them suitable for efficient qPCR, but preclude a single clear top-hit for taxonomy, therefore the top 100 hits for each sequence are summarized here.

[b]Lowest Common Ancestor (LCA) assignment was carried out in MEGAN5. While primers may be more specific than suggested by their class level taxonomic designation, no sequence produced any hits within the top 100 that fell outside of that class designation.

Supplemental Experimental Procedures

Fly culture and Lifespan

The majority of this work was carried out in the standard laboratory strains w^{1118} and Canton S. Additional genotypes used were the *5966* and *S106* GeneSwitch lines provided by L Jones and L Seroude respectively, and *UAS-toll10*^{b} and *UAS-PGRP-LC* provided by M Dionne. Flies were cultured in a humidified, temperature-controlled incubator with 12h on/off light cycle at 25 °C, in vials containing standard cornmeal medium (1% agar, 3% brewer's yeast, 1.9% sucrose, 3.8% dextrose and 9.1% cornmeal; all concentrations given in wt/vol). Adult animals were collected under light nitrogen-induced anesthesia, housed at a density of 27-32 flies per vial and flipped to fresh vials and scored for death every 2-3 days throughout adult life. RU486 (Cayman Chemical Company) to induce the geneswitch activity was dissolved in ethanol and mixed into the media when preparing food vials. RU486 doses used were 5 or 50 ug/ml final concentration and control food had ethanol alone, the volume of ethanol in each case was kept the same. Antibiotic treatment was conducted as described previously (Brummel et al., 2004). The Smurf assay, for barrier integrity, was carried out as described previously (Rera et al., 2012), except that flies were kept on the blue food for a 24 hour period before being scored. In every experiment, regardless of the conditions used, control and experimental animals are always transferred to fresh food at the same time-points. This provides an important control for bacterial growth in the food throughout these experiments.

Generation of axenic and re-associated flies

To generate axenic (germ-free) flies, embryos were treated by bleach and ethanol as described previously (Bakula, 1969). Briefly, <12-h-old embryos were dechorionated in 3% sodium hypochlorite (50% v/v regular bleach) for 20 min, rinsed in 70% ethanol for 5 min, and then washed three times with 1× PBS + 0.01% Triton X-100. Axenic embryos were transferred to autoclaved medium (500 embryos/bottle) in a laminar flow cabinet. Axenic conditions were confirmed by plating the fly homogenate on MRS agar. To generate flies associated with microbes as embryos, whole fly homogenate (10 fly equivalent: 600 μL of conventionally reared fly homogenate glycerol stock/bottle) was added to medium containing axenic embryos.

Preparation of fly homogenate for re-association and adult feeding

Conventionally reared adult flies were surface sterilized by 70% ethanol as previously described (Ren et al., 2007) prior to homogenization to ensure only internal microbes were present in the homogenate. Surface sterile flies were homogenized with a motor pestle in 1.5mL tube with 200 μL of sterile PBS (50 flies/tube). Homogenates were then pooled and sterile PBS added to adjust to one fly equivalent in 50 μL PBS. For storage 1/5 volume of 80% sterile glycerol was added and aliquots were stored at -80º C until use. For adult feeding, freshly prepared homogenate (one fly equivalent in 50μL PBS) was added to standard food vials and allowed to dry.

Fecal sampling

Fecal samples were collected similarly to previously described methods (Fink et al., 2013), but with some modifications. Vials containing food supplemented with Blue dye #1 were partially lined with aluminum foil, and an individual 40 day old w^{1118} female fly was added to each vial. Each day the aluminum foil was removed and stored at -80 ºC, and the fly was scored for Smurf status. The fly was then returned to a newly lined vial. The food vial was changed on the normal schedule (every 2-3 days). At the end of the 10 day time-course, the flies were grouped according to their Smurf status on day 7 or 9. Fecal samples for Smurf flies and a similar number of non-Smurf controls were first assessed for the number of fecal pellets, to enable normalization by deposit quantity. Fecal samples were then carefully swabbed with Sterile polyester tipped applicators (Puritan) wetted with sterile PBS, avoiding any food matter at the base of the foil. Foil collections from adjacent days were pooled for swabbing, so each swab collected fecal samples from a 48 hour period. Applicator tips were then broken off and stored in sterile eppendorf tubes at -80ºC prior to DNA extraction. To ensure that sufficient pre-Smurf fecal samples were available, and to ensure fecal collection was consistent relative to the food transfers (to control for feeding affects on bacterial load), flies that lost barrier function prior to day 7, or on day 8, were excluded from the assay.

Genomic DNA isolation

Genomic DNA was extracted using the PowerSoil DNA isolation kit (MoBio). All flies were surface sterilized as previously described (Ren et al., 2007) prior to sample preparation. To ensure consistent homogenization, whole fly samples were pre-homogenized in 150μL of solution from the PowerSoil bead tube using a motor pestle. This homogenate was then returned to the bead tube and the manufacturers protocol was followed. For intestinal samples, flies were surface sterilized in small

groups and then dissected over ice, in sterile PBS and with sterile equipment. The dissection surface was swabbed with 75% ethanol between each sample. Intestinal dissections included all but the anterior foregut, from the point at which the crop diverges, and including the crop, to the rectal papilla. Care was taken to keep the full length of the gut intact to prevent loss of lumen contents. Dissected intestines were stored in sterile eppendorf tubes at -80ºC prior to DNA extraction and were then pre-homogenized as described above. Fecal sample swabs were added directly to the PowerSoil bead tube, and the manufacturers protocol was followed.

Sequencing and Analysis

Genomic DNA samples from dissected intestines were prepared as described above. Indexed paired end libraries were generated with Nextera XT DNA sample preparation kit and Nextera XT Index kit (Illumina), with 1 ng of starting material and following the manufacturers protocol, but with an extended fragmentation time of 8 minutes. Average fragment length was 700 base pairs. The concentration of each library was quantified with the qubit HS assay. Concentration normalized libraries were then pooled and sequenced on Illumina HiSeq2000 with TruSeq Dual Index Sequencing Primer Kit, Single Read (Illumina). The quality of the sequence dataset was confirmed using fastQC software (Babraham bioinformatics). Over 10,000,000 reads from each sample were then searched against a 2014 version of the NCBI non-redundant database (nr) using RAPSearch version 2.18 (Zhao et al., 2012), limiting output to 50 matches per query. RAPSearch results were imported to MEGAN5 (Huson et al., 2011) for taxonomic analysis.

MEGAN utilizes the NCBI taxonomy and a lowest common ancestor assignment algorithm for taxonomic binning, whereby the taxonomic level at which a sequence is assigned reflects its level of conservation. This approach promotes unspecific assignments over false positives and allows resolution at all taxonomic levels. A bit score threshold is applied and any sequence alignment that falls below threshold is discarded (Huson et al., 2011). We retained the programs default settings for taxonomic binning. MEGAN also enforces a minimum level of support for each taxa and reports only taxa that are assigned over a minimum number of reads. Reads that are initially assigned to a taxon that is later not deemed present are not removed but are included at a higher taxonomic level. As an additional quality control step, to ensure that we were considering only taxa that are truly present in our samples, the main analysis presented here shows only those taxa that display abundance similar to, or greater than, that of the *Drosophila* endosymbiont *Wolbachia*, which was present in all of our samples. On average, 10,000 reads were assigned to *Wolbachia* across our samples. This therefore

represents a stringent abundance threshold. Where taxa that fall below this threshold are presented, this is clearly stated. Finally, in order to compare taxa abundance between samples square-root normalization for sequencing depth was applied by the MEGAN software (Huson et al., 2011)

Fecal analysis

Fecal plates for analysis were prepared as described previously (Cognigni et al., 2011); however, flies were initially placed on food containing Blue dye #1 in order to assess Smurf status. In order to ensure that Blue dye #1 did not confound the fecal analysis, the flies were then given 24 hours on food containing 0.5% Bromophenol blue sodium salt (B5525, Sigma) before being transferred to assay plates. The assay was run for 24-48 hours as indicated. Plates were scanned on an Epson Perfection v750 pro transparency scanner at 1200 dpi. Images of plate bottoms were cropped out of the initial imaging file, ensuring that selection size was consistent, using Fiji (Schindelin et al., 2012) and were then processed and analyzed in The Ultimate Reader of Dung (T.U.R.D) as previously described (Wayland et al., 2014).

Feeding assay

Analysis of capillary feeding ("CAFE" assay) was performed as previously described (Deshpande et al., 2014; Ja et al., 2007). Feeding was monitored for a 24 hour period.

Immunostaining Procedure for Anti-discs large

30 day old female flies were sorted for Smurf status and transferred to standard food to clear the blue dye. After 5 days, the flies were anesthetized on ice, and intestines were dissected in cold PBS. Samples were then fixed in 4% formaldehyde in PBS at room temperature for 1 hour and rinsed four times in PBS + 0.5% Triton X-100 (PBS-Tx) for 10 min. Blocking was performed in 3% BSA in PBS-Tx. Primary antibody, mouse anti-discs large (anti-dlg from the *Drosophila* Hybridoma Bank, 4F3) was added at 1:15 and incubated overnight at 4°C. Samples were then rinsed four times in PBS + 0.5% Triton X-100 for 10 minutes at room temperature. For the secondary antibody incubation, anti-mouse AlexaFluor-488 (Invitrogen) was added 1:200 and To-Pro-3 DNA stain (Invitrogen) 1:200 in 3% BSA in PBS + 0.5% Triton X-100 for two hours at room temperature. Samples were rinsed four times in PBS + 0.5% Triton X-100 for 10 minutes at room temperature. Intestines were then mounted in Vectashield mounting medium (Vector Labs) and imaged using Zeiss single point LSM 5 exciter confocal microscope. For anti-discs large intensity quantifications, Z stacks of the enterocytes of the

posterior midgut were taken using identical settings with the 63X objective. Relative intensity was calculated using the ratio of integrated density of anti-dlg staining to the integrated density of TO-Pro-3 staining from a set area of 360 μ m2 for each midgut. Statistical analysis was conducted on the mean relative intensity using a Mann-Whitney U test implemented in R v2.14.2 (n>20 guts were used per condition).

Quantitative PCR

DNA samples for qPCR of the 16S ribosomal RNA gene were prepared as described above. RNA extractions, for gene expression analysis, were carried out in TRIzol (Invitrogen) following the manufacturer's directions. Intestinal dissections for RNA extraction were as described for DNA isolation but without the sterilization steps. cDNA synthesis was carried out using the First Strand cDNA Synthesis Kit from Fermentas. PCR was performed with *Power* SYBR Green master mix (Applied Biosystems) on an Applied Biosystems 7300 Real Time PCR system. Cycling conditions were as follows: 95ºC for10 minutes; 95ºC for 15s then 60ºC for 60s, cycled 40 times.
All calculated gene expression values were normalized to the value of the loading control gene, Actin 5C. The primer sequences used to assess gene expression in this study were as follows: Act5C_L – TTGTCTGGGCAAGAGGATCAG, Act5C_R - ACCACTCGCACTTGCACTTTC; Dro_L – CCATCGAGGATCACCTGACT, Dro_R – CTTTAGGCGGGCAGAATG; Drs_L – GTACTTGTTCGCCCTCTTCG, Drs_R – CTTGCACACACGACGACAG; Dpt_L – ACCGCAGTACCCACTCAATC, Dpt_R – CCCAAGTGCTGTCCATATCC; Mtk_L – TCTTGGAGCGATTTTTCTGG, Mtk_R – TCTGCCAGCACTGATGTAGC; Duox_L – GGGAGTCTTATGGACTGAAAC, Duox_R GTACGCCTCCTTCAGCATGT; upd3_L – GCAAGAAACGCCAAAGGA, upd3_R – CTTGTCCGCATTGGTGGT; Socs36E_L – AAAAAGCCAGCAAACCAAAA, Socs36E_R – AGGTGATGACCCATTGGAAG; DEcad_L – GACGAATCCATGTCGGAAAA, DEcad_R – TCACTGGCGCTGATAGTCAT; delta_L - AGTGGGGTGGGTGTAGCTTT, delta_R – GCTGTTGCTGCCAGTTTTG; Notch_L – GAATTTGCCAAACACCGTTC, Notch_R – ACCGACACTTGTGCAGGAA; pck_L – GCTCTCGCTTACCATCATCC, pck_R – TACGGCCAAAAACATGAACA; Kune_L – AGGTTGTGGGCTCTGTTTTC, Kune_R – ATCCCGAGAATCTCCTTTGG; sinu_L – CATTGAATTGCATAAACTTCAGCTA, sinu_R – GCGGAGTTTCGCTTACCTT; pyd_L – TGAATCGAGAGGCAACTTCTT, pyd_R – TTCTCGCGGGACAGACTC; dlg1_L – AGAGTCGCGATGAGAAGAATG, dlg1_R – GCTGGTGCTGCTCACAACT.

Universal primers for the 16S ribosomal RNA gene were against variable regions 1 (V1F) and 2 (V2R), as previously published (Claesson et al., 2010). Taxon specific 16S primers were as follows: Bacilli_F – CGACCTGAGAGGGTAATCGGC, Bacilli_R – GTAGTTAGCCGTGGCTTTCTGG; Alpha_F – CCAGGGCTTGAATGTAGAGGC, Alpha_R – CCTTGCGGTTCGCTCACCGGC; Gamma_F – GGTAGCTAATACCGCATAACG, Gamma_R – TCTCAGTTCCAGTGTGGCTGG.

16S cloning and analysis

All 16S primers detailed above were run in a standard PCR with Phusion Hot-Start DNA polymerase (New England Biolabs) and a 96 hour post-SMF intestinal genomic DNA extraction as template, or a no template control. Cycling conditions were as follows: 98ºC for 30 seconds; 98ºC for 7s then 60ºC for 20s then 72ºC for 30s, cycled 40 times; final extension was 72ºC for 5 minutes. PCR products were cloned using the Zero Blunt TOPO PCR cloning kit for sequencing (Invitrogen), following the manufacturers protocol. 24 colonies were picked for each condition into 20μL of sterile ddH_2O and lysed at 94ºC for 10 minutes. 5μL of each lysed colony was then used as template for PCR amplification using the standard M13 forward and reverse primers, supplied with the cloning kit, again following the manufacturers protocol. PCR clean-up and sequencing were carried out by Laragen, Los Angeles, CA. Vector sequences were trimmed from the resulting sequence reads and reads with 100% identity were aligned in a single consensus. Each consensus sequence was then compared with the NCBI 16S ribosomal RNA database using BLASTN 2.2.30+ and the top 100 hits for each sequence were retained for taxonomic analysis. Any apparent chimeric sequences were excluded from further analysis. Lowest common ancestor assignments were carried out by MEGAN5 as detailed above.

Statistics

The comparison of survival curves was done using the log-rank test as implemented in the Graphpad Prism software. Comparison of Smurf proportion per time point was carried out using a binomial test to calculate the probability of having as many Smurfs in population B as in population A. All other data comparisons were tested for significant differences using the Wilcoxon-Mann-Whitney U test where sample sizes were greater than five, and a Student T test where sample sizes were fewer than five. The number of biological replicate samples is given in each figure legend. All statistical tests, except the log-rank, were implemented in R v2.14.2. All statistical tests are two-sided.

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